CSP Manager,
Joint Genome Institute
2800 Mitchell Drive
Walnut Creek, CA 94598

Dear CSP Manager,

Hereby our response to your request for a written statement declaring that the DNA sample we provided is free of living material. The DNA provided by me to the CSP of JGI of *Mycosphaerella graminicola* isolate 323 characterised by the strain name CBS 115943 is free of living material.

The starting material was a pure culture of *Mycosphaerella graminicola* and the DNA isolation procedure we used kills all *Mycosphaerella graminicola* (See the appendix which contains a detailed description of the procedure and describes the steps that kill living material of *Mycosphaerella graminicola*). As recommended, we confirmed that *Mycosphaerella graminicola* did not survive the procedure by culturing part of the 10 µl of the DNA sample on plates with Potato Dextrose Agar (PDA) the regular medium to grow *Mycosphaerella graminicola*. No growth of the DNA sample was detected whereas abundant growth of *Mycosphaerella graminicola* was found on control plates, inoculated with *Mycosphaerella graminicola* spores (see figures in the appendix).

With kind regards,

As a sterility test, the collaborator typically plates 5-10% of the DNA prep and grows it under the appropriate conditions for the specific organism.

The sterility document can be emailed. The document must include:

- Full name of the source organism (Genus Species Strain)
Mycosphaerella graminicola strain 323 (CBS)

- Point-of-contact information including organization, affiliation, address, phone numbers, email.

Name: [redacted]
Organisation: [redacted]

Business Unit: [redacted]
Address: [redacted]
Postal Code: [redacted]
City: [redacted]
Country: [redacted]
Cell phone: [redacted]
Office Phone: [redacted]
Home phone: [redacted]
Fax: [redacted]
E-mail: [redacted]
Building: [redacted]
Room: [redacted]
Street: [redacted]
Postal code: [redacted]
City: [redacted]
Country: [redacted]

- A description of the method of culture (with references), the procedure used to sterilize the material, and method of verifying sterilization.

DNA Isolated from *Mycosphaerella graminicola* contains no viable spores due to the procedure of DNA isolation described below.

Preparation of the spores:
Some mycelium was used to inoculate YG Broth (Yeast Extract 10g/l, Glucose 30 g/l) and the culture was incubated by shaking at 120 rpm at 18℃ for 5 days. Spores were then left to settle overnight and were lyophilised for at least 1 day.
The DNA isolation method used for the extraction was the **Promega Wizard Magnetic DNA Purification System for Food** of which the first two steps were slightly modified. This DNA purification method relies on the binding of DNA (&RNA) to tiny magnetic beads with surface properties similar to the silica resins commonly used in other DNA & RNA purification kits.
Preparation of the genomic DNA:
1. 50 mg of lyophilised spores was placed in a 2mL tube.
2. The lyophilised spores were grinded using the Hybaid Ribolyser (model n°FP120HY-230) for 10 s at 2500 rpm with a tungsten carbide bead. 500µL of Lysis Buffer A and 5µL of

RNase A were added and sample was again submitted to homogenisation using the Hybaid Ribolyser and the same conditions.
3. 250µL of Lysis Buffer B was added and the sample was mixed using a vortex for 10–15 seconds. After which the tube was incubated for 10 minutes at room temperature (22–25℃).
Note: Extraction of DNA relies on breaking open spores at this point. Mechanical damage with tungsten carbide beads and vigorous shaking in lysis buffers (buffer B contains Guanidinium Thiocyanate, a potent denaturant and chaotrope, to improve lysis) destroys most spores here.
4. 750µl of Precipitation Solution was added and the sample was mixed vigorously on a vortex.
5. The tube was centrifuged for 10 minutes in a microcentrifuge at maximum speed (13,000 × g).
6. The supernatant (liquid phase) was transferred to a new 2ml tube.
Note: Centrifugation at this speed effectively pellets debris from lysed cells as well as still-viable spores. Very few viable spores (if any) should be carried over as long as the pellet remains intact and the researcher does not attempt to remove all of the liquid from the old tube to put into the new tube.
7. 50µl of a thoroughly resuspended MagneSil™ PMPs was added to the supernatant and mixed with a vortex.
8. Approximately 0.8 volume of isopropanol was added and the tube was inverted 10–15 times. The sample was left to incubate for 5 minutes at room temperature with occasional mixing.
Note: The MagneSil PMPs are para-magnetic particles and specifically bind DNA & RNA. Proteins and spores will not stick to the surface of the MagneSil PMPs.
9. The tube was placed onto the MagneSphere ® Magnetic Separation Stand and left for 1 minute after which the liquid phase was discarded.
Note: in this step the beads are pulled to the side of the tube by a strong magnet, while the liquid – containing dead, as well as some still viable, spores is removed.
10. The tube was removed from the stand and 250µL of Lysis Buffer B was added. The tube was inverted 2–3 times and placed back in the stand. After 1 minute to allow the MagneSil™ PMPs to separate the liquid phase was removed.
11. 1ml of wash solution was added to the sample and tube was placed on the stand for 1 minute. The liquid waste was discarded. This washing was performed three times.
Note: after these four washes – once with more lysis buffer and thrice with a salt/ethanol/water solution – the DNA bound to the beads should be completely free of any remaining viable spores.
12. The particles were dried for 10 minutes at 65℃.
13. 100µL of sterile water was added to the particles (1st elution) and the sample was mixed with a vortex and incubated at 65℃ for 5 minutes. The tube was placed onto the magnetic stand and the liquid was transferred to a new micro-tube.

- Negative culture results with positive control.

2 times 10 µl of the DNA sample was plated on PDA agar and at the same time 10 µl of a spore suspension of *Mycosphaerella graminicola* strain 323 (CBS) was plated on PDA as a possitive control. After six days of incubation at 20 ^{o}C the plates were chequed. No grow was found on the plates inoculated with the DNA samples. A picture was taken from the plates. See the attached figure.

- A statement in writing from the individual providing the DNA that the sample(s) is pathogen free.

The starting material was a pure culture of *Mycosphaerella graminicola* strain 323 (CBS) and the DNA isolation procedure we used kills all *Mycosphaerella graminicola* (See the appendix which contains a detailed description of the procedure and the steps that kill of living material of *Mycosphaerella graminicola)*

Negative culture results with positive control. Ten microliter of DNA was plated on PDA and grown for 6 days at 20°C (plates on the right side). Abundant growth of *Mycosphaerella graminicola* was found on control plates inoculated with *Mycosphaerella graminicola* spores (plates on the left).